# RADIOCOBALT ACCUMULATION IN TETRAHYMENA

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Previous studies have indicated that cobalt is essential for growth in *Tetrahymena* (Slater, 1952; Roth, 1956), but there is no information available about cation uptake in this animal. Although the major function of cobalt is believed to be that of serving as part of the vitamin $B_{12}$ molecule (Marston, 1952), cobalt is also implicated as the element *per se* in important hydrolytic reactions (Johnson and Berger, 1942).

The present experiments were designed to study the accumulation of cobalt in protozoans during growth. Elucidation of some of the factors regulating cobalt transport between the medium and the organism was also attempted. This study included the growth phase, the influence of deficient medium on exchange, the influence of population density on uptake per animal, and the effect of ion concentration on uptake.

## Materials and Methods

Strain E of *Tetrahymena pyriformis* was used in this investigation, and all the experiments were performed in synthetic medium (Slater, 1952). Calcium, uracil, and adenylic acid were omitted from the media in all instances and cytidylic and guanylic acids were reduced to 10 $\mu$gm./ml. levels. In some experiments, growth effects were eliminated by use of media deficient in essential growth factors.

Cobalt-60 was used as a tracer and adjusted to 0.1 $\mu$c./ml. (final concentration) except where indicated. Cultures were grown in 10 ml. of synthetic medium in 18-mm. Pyrex tubes, and growth was measured turbidimetrically with a Lumetron (Model 400) colorimeter equipped with a red (650 m$\mu$) filter. Radiations were detected with a deep-well scintillation detector and a Nuclear Instrument and Chemical Corporation Scaler (No. 162). Constriction chamber centrifuge tubes enabled clear separations of organisms from supernatant upon mild (100 G, one minute) centrifugation. The culture was washed with non-radioactive synthetic medium to remove excess fluid. The histidine in this medium is known to form a strong complex with cobalt (Burk *et al.*, 1946).

Prior to the introduction of $Co^{60}$, no cobalt was detected in the synthetic medium by ultraviolet emission spectroscopy, the porous cup technique being used. One

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liter of synthetic medium was concentrated 500-fold by evaporation for this analysis, and it was estimated that less than 0.01 μgm./ml. of cobalt ion was present.

Radiation effects from the tracer used have, in many instances, been known to influence physiological processes. The extreme resistance of *Tetrahymena* to radiation (Elliott and Slater, 1951), however, makes it unlikely that any influence from the tracer's radiation was significant during these experiments.

After the protozoans were separated from the supernatant by centrifugation, they were placed in two-ml. volumetric tubes and adjusted by micropipettes to 2.0-ml. volumes with distilled water. They were then transferred quantitatively to

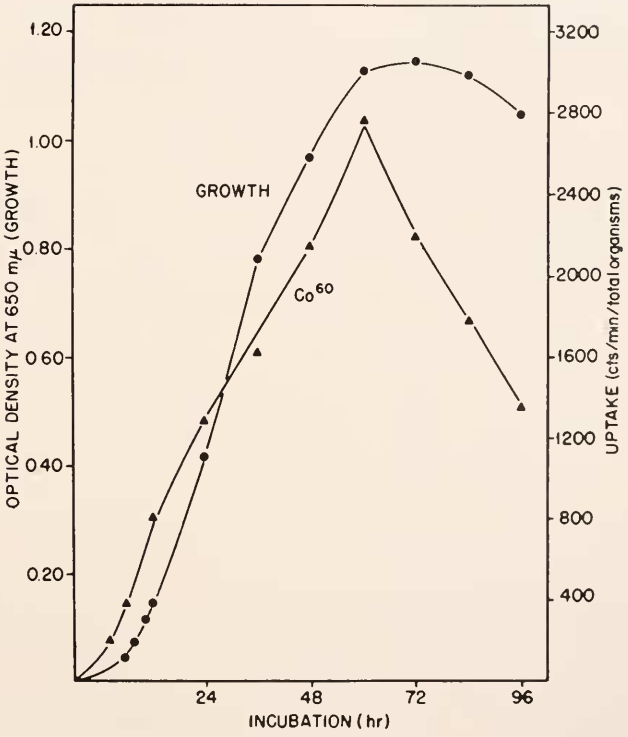


FIGURE 1. Cobalt-60 uptake and release during growth in *Tetrahymena*.

plastic tubes. Since the volume of the column of radioactive substance materially affected the number of counts registered by the scintillation detector, exactly 2.0-ml. volume adjustments were used throughout.

## RESULTS

### *1. Uptake during growth*

In the first series of experiments, $Co^{60}$ at 0.01 μc./ml. (final concentration) was introduced into each culture at the beginning of the experiment as a tracer for the movement of this element during growth. Spectrographic analysis revealed that the

added cobalt amounted to 3.7 μgm./ml. (final concentration). No growth effects were noticed from cobalt at this concentration in preliminary experiments.

There was a steady uptake of cobalt during growth and an abrupt release of this ion shortly after the stationary phase was reached (Fig. 1). The temperature in the typical experiment reported was 27.5° ± 0.5° C., and the initial inoculum from mid-log phase cultures amounted to 185,000 ± 5% animals per tube. The total uptake during 60 hours of growth amounted to 0.97 μgm. of Co per total mass of cells, or about 26% of the cobalt present. This amounted to $4.6 \times 10^{-8}$% of the total cobalt present per hour per organism. Further calculations revealed that each *Tetrahymena* at 60 hours possessed about $10^9$ atoms of cobalt. The number of animals remained constant from the sixth to the twelfth hour after inoculation (Table I) although the optical density measurements increased steadily. Uptake of cobalt during this period was probably associated with the increase in volume of the individual

TABLE I

*Cobalt-60 uptake during growth for the first 12 hours*

| Time (hr.) | Optical density at 650 mμ | Number of animals | Uptake for total population (cts./min.) |
|---|---|---|---|
| 0 | | 185,000 | |
| ¼ | | | 77 |
| 2 | | | 208 |
| 4 | | | 252 |
| 6 | 0.04 | 479,000 | 381 |
| 8 | 0.07 | 459,000 | 489 |
| 10 | 0.11 | 457,000 | 743 |
| 12 | 0.14 | 466,000 | 806 |

organism. The volume of *Tetrahymena* reaches a maximum near the upper third of the log phase and falls off to about one-half this value upon reaching the stationary phase (Slater and Elliott, 1951).

The release of $Co^{60}$ during the stationary phase was studied for only two days to avoid the possibility of measuring cobalt release from disintegrating cells. Microscopic observation of the protozoans during this period failed to reveal any obvious morphological breakdown, although an imperceptible physiological breakdown is certainly not an impossibility. Fifty per cent of the accumulated cobalt was released into the medium in 36 hours with a rate amounting to $1.4 \times 10^{-6}$%/hour/organism.

*2. Effect of number of animals on uptake*

The influence of number of animals on uptake per animal was studied. Eight-hour periods of time were selected to minimize growth effects and any influence from the accumulation of metabolic wastes. The number of animals present had a definite effect on uptake (Fig. 2) per animal. Populations of the order of $10^4$ animals became nearly ten times as radioactive as populations of $2 \times 10^6$ organisms. At these high population densities it is not improbable that there was a great deal of competition for oxygen and also that harmful metabolic wastes resulted in inhibitory effects. Population densities of $2 \times 10^5$ cells, however, were far from being crowded under the experimental conditions and yet contained only one-third the ac-

tivity of the lowest concentration. In the experiment shown, the temperature was maintained at 26.5° ± 0.5° C., and cobalt was introduced at the 0.01 μc./ml. level.

*3. Cobalt release in deficient medium*

Release of cobalt was studied in media deficient in essential growth factors, salts, and glucose. Log-phase cultures were allowed to incubate initially in complete synthetic medium containing 0.01 μc./ml. of $Co^{60}$ for 24 hours. The animals were

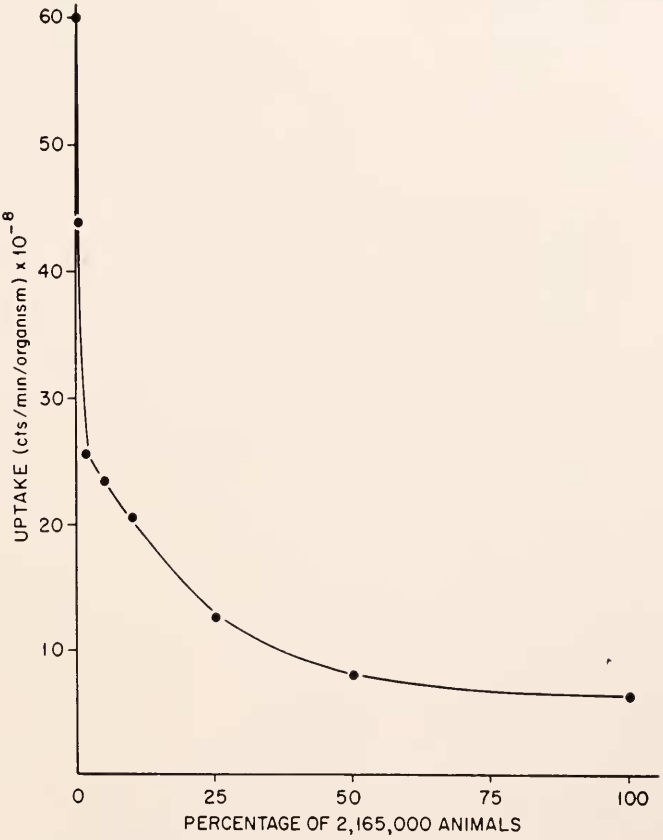


FIGURE 2. Influence of number of animals on uptake per animal.

then removed by centrifugation, washed once with deficient medium, and resuspended in deficient medium. This medium was used to prevent growth effects. In the typical experiment illustrated (Fig. 3) the initial population amounted to 415,000 cells/tube. These had grown to about twice this number at the time of introduction to the "cold" medium.

Two mechanisms are evident during cobalt release under these conditions. The first is very rapid and takes place within two hours. The rate of release during this time was 13%/hour/organism. The second mechanism is much slower and

amounts to 1.7%/hour/organism. Under these conditions, 50% of the cobalt was released in about 20 hours.

*4. Cobalt concentration ability*

The ability of protoplasm to concentrate certain elements has been known for a long time. *Tetrahymena* is no exception in this capacity. In the first series of experiments, uptake in relation to cobalt concentration was studied during 12 hours.

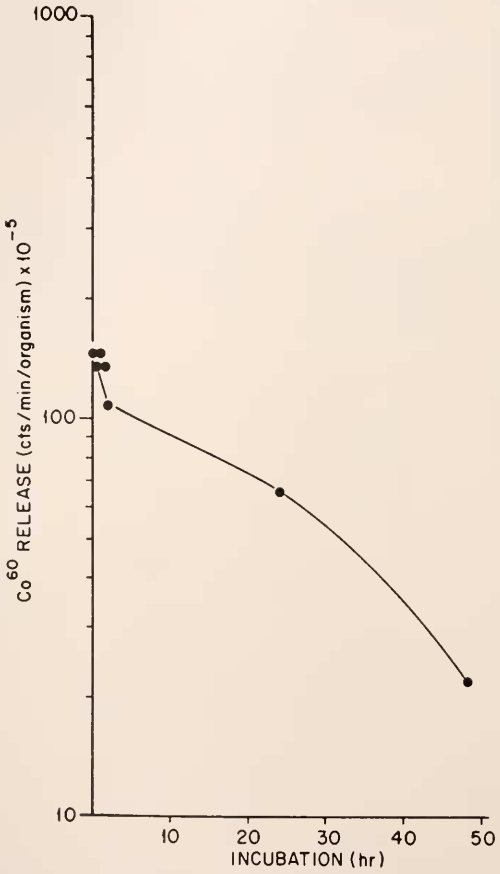


FIGURE 3. Release of cobalt-60 in deficient medium.

Complete synthetic medium was used in all instances, and the relatively short time interval was used to minimize growth effects. The concentrations used varied from 0.0005 $\mu$c./ml. of $Co^{60}$ ($3 \times 10^{-6}$ *M*) to 0.0100 $\mu$c./ml. of $Co^{60}$ ($6 \times 10^{-5}$ *M*). Uptake during 12 hours' incubation was found to be directly proportional to concentration (Fig. 4). The animals concentrated cobalt to the extent of 4.4–5.7 times that present in similar volumes of the environment regardless of the amount of isotope present (Table II). The volumes of individual animals used in these calculations are adapted from data presented earlier (Slater, 1951).

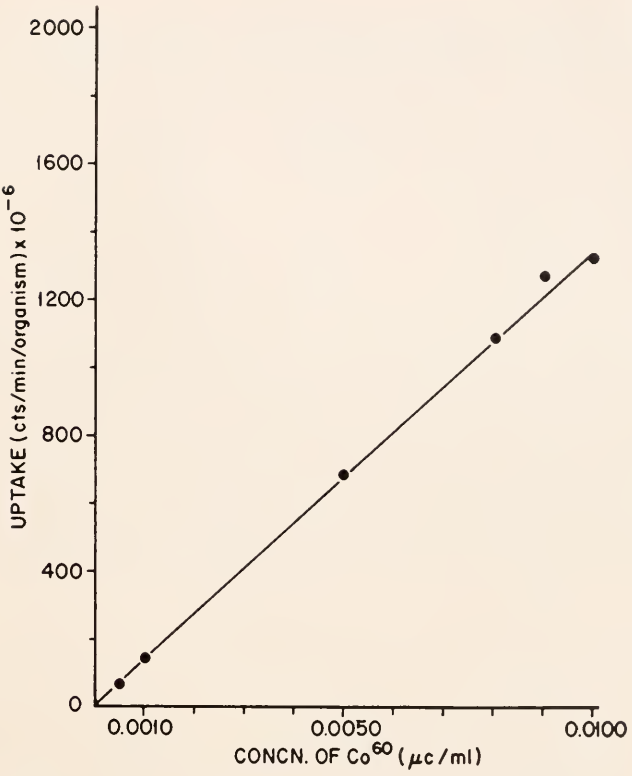


FIGURE 4. Effect of cobalt-60 concentration on 12-hour uptake.

TABLE II

*Influence of cobalt concentration on uptake in 12 hours*

| Conc. of $Co^{60}$ ($\mu$c./ml.) | $Co^{++}$ ($\mu$gm./ml.) | Cts./min./total population | Cts./min./vol. occupied by population* | Degree of conc.** cts./min./total pop. / cts./min./vol. occup. |
|---|---|---|---|---|
| 0.0100 | 3.70 | 1104 | 221 | 4.9 |
| 0.0098 | 3.63 | 942 | 216 | 4.4 |
| 0.0096 | 3.55 | 1211 | 212 | 5.7 |
| 0.0094 | 3.48 | 1126 | 207 | 5.4 |
| 0.0092 | 3.40 | 1169 | 203 | 5.7 |
| 0.0090 | 3.33 | 1062 | 198 | 5.4 |
| 0.0080 | 2.96 | 910 | 176 | 5.2 |
| 0.0050 | 1.85 | 573 | 110 | 5.1 |
| 0.0010 | 0.37 | 117 | 22 | 5.3 |
| 0.0005 | 0.185 | 58 | 11 | 5.3 |

* Calculated from $\mu$c./ml. times cts./min./$\mu$c. times volume occupied by protozoans after 12 hours growth. One ml. containing 0.01 $\mu$c. of $Co^{60}$ gives 10,500 cts./min. The volume occupied by these populations equaled 0.0212 ml. after 12 hours growth.

** Representing the concentration of cobalt by the entire population of animals over that contained in comparable volumes.

TABLE III

*Influence of cobalt concentration on maximum uptake*

| Conc. of $Co^{60}$ ($\mu$c./ml.) | $Co^{++}$ ($\mu$gm./ml.) | Max. uptake cts./min./total population | Cts./min./vol. occupied by population* | Degree of conc. cts./min./total pop. / cts./min./vol. occup. |
|---|---|---|---|---|
| 0.01 | 0.764 | 2064 | 265 | 7.8 |
| 0.005 | 0.508 | 1373 | 132 | 10.4 |
| 0.001 | 0.148 | 399 | 27 | 14.8 |
| 0.0005 | 0.061 | 165 | 13 | 12.7 |
| 0.0002 | 0.025 | 68 | 5 | 13.6 |

pH, 7.4; temperature, 25.7° ± 0.5° C.

* Calculated from $\mu$c./ml. times cts./min./$\mu$c. times volume occupied by protozoans at maximum uptake. One ml. containing 0.01 $\mu$c. of $Co^{60}$ gives 10,500 cts./min. The volume occupied by these populations equaled 0.0253 ml. of maximum uptake (Slater, 1951).

The influence of cobalt concentration on maximum uptake during growth was also studied. The greatest absolute amount of this ion was taken up by cultures in the presence of 0.764 $\mu$gm./ml. of $Co^{++}$ (Table III). The ability to concentrate this ion over that contained in the medium, though, reached maximum at 0.1–0.5

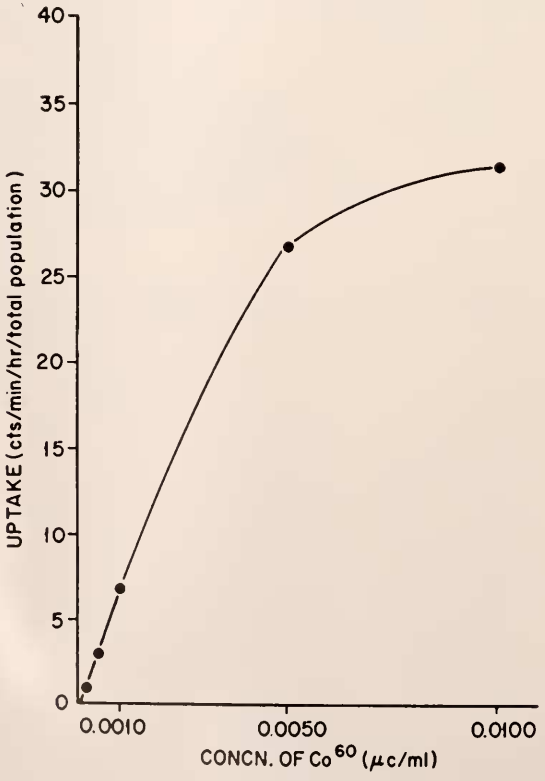


FIGURE 5. Uptake of cobalt-60 with concentration at 36 hours.

$\mu$gm./ml. of $Co^{++}$. The maximum uptake was about one and three quarters times that with 0.764 $\mu$gm./ml. of $Co^{++}$. Correspondingly, the rate of uptake seemed to be linear with concentrations up to 0.508 $\mu$gm./ml. of $Co^{++}$ (Fig. 5) but was less at higher concentrations.

## Discussion

The uptake of cations by animal cells involves a complex spectrum of interrelated processes, any one of which may alter the total cellular absorptive capacity. Among these controlling factors are the rate of utilization and probably intracellular translocation. Probably the uptake of inorganic substances first involves a combination with organic cell constituents (Sutcliff, 1954) ; specific metabolic pumps may also be involved. These cations then may become distributed to different cellular sites in response to varying chelation forces, which appear as the environmental mixture of cations changes. Thus during periods of major synthesis and growth, forces primarily concerned with cell divisions and external membrane changes might be most active, while during periods of relative inactivity, forces concerned with exchange, turnover, and simple release might well become more active. Chelation forces may also be involved in mitochondrial physiology and are probably involved in ion transport in these particulates. Studies of the relative binding abilities of various sites within the cell must certainly be done if the intricacies of cation transport and translocation are to be elucidated.

In *Tetrahymena*, it was shown that the population density may greatly influence the uptake of cobalt per cell. At great densities, cellular chelate forces may compete for environmental cations. It is also considered likely that competition for dissolved nutrients in general may inhibit the transport mechanism for any given cation.

It was demonstrated earlier that cobalt was essential for growth in purified synthetic medium without glucose. The physiological role of this cation in protozoans where an extraneous source of the $B_{12}$ molecule is not required is not clear. It has been suggested that cobalt ion in these experiments may act non-specifically to increase the availability of other cations by releasing them or displacing them from complexes (Ford and Hutner, 1955, pp. 101–136) at the cell surface (Hutner *et al.*, 1950). Since Roth (1956) has shown that nickel does not have the same effect as cobalt on growth in *Tetrahymena*, and since the present experiments have demonstrated that cobalt is differentially bound to the animal, depending on the concentration of the cation, any non-specific effect may be minimal.

The uptake of cobalt during growth has been studied in *Bacillus subtilis* (Tanaka *et al.*, 1952), *Neurospora crassa* (Ballentine and Stephens, 1951), and *Saccharomyces cerevisiae* (Nickerson and Zerahn, 1949). No studies involving protozoan uptake of this ion seem to have been published.

Sizable losses of cobalt were observed with advancing age of the population in all the above organisms. In both *Tetrahymena* and *B. subtilis*, the release of the ion began abruptly after the stationary phase began. A comparison of the concentrating abilities of *Saccharomyces* and *Tetrahymena* showed that *Saccharomyces* had nearly 70 times the ability to concentrate cobalt possessed by the protozoan. Two washes of *Tetrahymena* did not appreciably remove the ion, and over 20 hours' washing of *Saccharomyces* also showed that cobalt was firmly held. Nickerson and Zerahn (1949) suggested that the presence of peripherally located metaphosphate might be significant in the accumulation of "metals" from dilute solution by yeasts.

In *Neurospora* (Ballentine and Stephens, 1951), at least 40% of the cobalt accumulated was present in stable cobalto-proteins. Fractionation of these compounds revealed the presence of a soluble fraction comprising 57% of the stably bound cobalt and a nearly submicroscopic particulate fraction. Similar cobalto-proteins were also found in *Chlorella vulgaris* and in the leaves of the musk melon and tomato. As with *Neurospora, Saccharomyces,* and *B. subtilis, Tetrahymena* concentrated cobalt against a concentration gradient.

Scott and Ericson (1955) reported that cobalt was absorbed by the marine alga, *Rhodymenia palmata,* and became bound within the plant as a complex quite different from $B_{12}$. Analysis of this complex revealed the presence of several components. Thus cobalt may play a multiphysiological role in protoplasm. Ericson (1952) reported that sea weed possessed an unusual ability to absorb and concentrate $Co^{60}$ but the absorption of vitamin $B_{12}$ was very limited and could not account for the concentration of the cation. In earlier work on the essentiality of cobalt for growth in *Tetrahymena* (Slater, 1952), it was shown that a definite response could be obtained in purified synthetic medium when as little as 0.5 μgm./ml. of cobalt ion was present. Under the conditions of those experiments, this amount of cobalt was equivalent to about $5 \times 10^{10}$ atoms of cobalt per animal. In the present experiments, nearly $10^9$ atoms of cobalt were accumulated per animal, presumably for non-growth purposes since growth progressed in controls without added cobalt. Thus, in purified synthetic medium, when extraneous cations are removed to a large degree, nearly 50 times as much cobalt is used for growth as is accumulated when growth proceeds under the influence of other cations.

In a study on cobalt localization in pooled white mouse cells, Rosenfeld and Tobias (1951) reported that most of the element was present in the cytoplasm and about 1% of it was firmly bound to cellular protein. Very little was discovered in the nuclei. Most of the cytoplasmic association was with the globulin in the bound fraction. The slowly released fraction of cobalt in *Tetrahymena* (Fig. 3) may be associated with a firmly bound fraction of this type, but this fraction appears to be about 75% of the total. The intracellular particulate localization of cobalt remains to be elucidated.

## Summary

1. A steady uptake of radioactive cobalt was observed during the growth of *Tetrahymena* in synthetic medium. When the initially-added amount of cobalt was 3.7 μgm./ml., nearly 26% of the total was accumulated during 60 hours of growth. This amounted to approximately one billion atoms of cobalt per animal.

2. Upon reaching the stationary phase, sizable amounts of cobalt were released from the population. Fifty per cent release was observed in 36 hours with a rate amounting to $1.4 \times 10^{-6}$%/hour/organism.

3. The number of animals present had a definite effect on the accumulation of cobalt per animal. Populations of $10^4$ animals became nearly ten times as radioactive as populations containing two million organisms.

4. In nutritionally-deficient media, the release of cobalt was biphasic. The first was very rapid and took place within two hours. The initial rate of release of cobalt amounted to 13%/hour/organism. The second mechanism was much slower and amounted to 1.7%/hour/organism. Fifty per cent release was noticed in 20 hours under these conditions.

5. As the concentration of cobalt was varied, the rates of uptake increased rapidly. Concentrations higher than 1.85 μgm./ml. had little effect. The greatest absolute amount of cobalt was accumulated when 3.7 μgm./ml. was initially present in the medium, but the ability to concentrate this ion over that contained in the environment reached a peak at 0.37 μgm./ml. of cobalt ion.

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